mutations per 400 bp. Conclusion: Error prone PCR has several advantages in improving the enrichment of the library. The method is rapid and the rate of mutation can be varied with controlling the content of the reaction mixture.
Keywords: Random Mutant Library, ureC Subunit, *Helicobacter Pylori*, Error-prone PCR

275. Cloning and Expression of an Inherent Inhibitory Peptide for TGF-Beta Signaling
*Mosayebzadeh H, Sankian M
Immunochemistry research lab, Immunology research centre, Buali research institute, Mashhad University of medical sciences, Mashhad, Iran

Background: Smurf2 is a member of the E3-ubiquitin ligase family of proteins. Its role is in inhibition of TGF-beta signaling cascade. A tryptophan rich sequence by the name of WW2/WW3 within the molecule is accountable for the binding of the molecule to Smad7. This final complex is the active form which can practically play its part in the inhibition. As depicted, it is clear that WW2\WW3 sequence is an essential component to take part in the negative regulation of the signaling, since, it provides a bridge for the assembly of the two molecules into one single active complex. Materials and Methods: The total RNA was extracted from human PBMC and was *reverse* transcribed into *cDNA*. The sequence of interest encoding the peptide was amplified using PCR primers containing TAT sequence at the 5'-end of forward primer (Because the final aim of this research project is to explore the impact of ww2\ww3 on TGF-beta signaling in living cells, it must enter into cells and for this purpose, tat peptide provides the means) and then the amplified region was inserted into expression vector PGEX-2. Recombinant vector was transformed into BL-21 codon plus for expression. After expression, the bacteria were lysed and the solution dialyzed. Finally, the peptide under investigation was purified by GST-tag chromatography and analyzed with SDS-PAGE and western blotting. Results: WW2/WW3-tat peptide was successfully expressed in codon plus bacteria. The peptide WW2\WW3 along with the GST-tag has a molecular weight of 36 KD which corresponds to the position of the peptide band in SDS-PAGE. Additional confirmation was carried out by western blotting against tat peptide with positive results. Conclusion: Ww2\ww3 peptide can be cloned and expressed in codon plus prokaryotic cells and can be separated using GST-tag chromatography so that it can be later examined for its perturbation on the signaling cascade.
Keywords: Cloning, Inherent Inhibitory Peptide, TGF-Beta

276. Computational Prediction for the Binding Affinity of Interleukins 3 and 5 and GM-CSF to Cell SurfaceReceptors on Human Eosinophils
Gavanji Sh[1,2], MohabatkarH[1] , GhaediK[2,3], Dormiani K[2], LachinaniL[2], Foruzanfar M[2], Nasr Esfahani M.H[2]
[1]Department of Biotechnology, Faculty of Advanced Sciences and Technologies, University of Isfahan, Isfahan, Iran, [2]Department of Molecular Biotechnology, Royan institute for Animal Biotechnology, Cell science research Center, ACECR, Isfahan, Iran, [3]Biology Department, School of Sciences, University of Isfahan, Isfahan, Iran

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a glycoprotein with molecular weight of 14.477 kD comprise 144 amino acids resdiues. The encoding gene of this glycoprotein is located on chromosome 5 in human. This protein stimulates proliferation and differentiation of macrophages. N-terminally seventeen amino acids are serving as a signal peptide and the rest of 127 amino acids are known as molgramostim. Data have revealed a high affinity of this protein for binding to a heterodimer receptor on surface of the cell. The respective receptor includes α and β chains which the β chain is similar to interleukins 3 and 5 receptors. Due to this similarity, interleukins 3 and 5 are able to compete with GM-CSF in binding to the receptor. In the present study, to compare binding affinity of interleukins 3 and 5 and GM-CSF to the related receptor, a computational prediction study carried out using Modeller, Hex and Molegro softwares. According to the results, interleukin 3 with -517.09 kJ/mole, interleukin 5 with -538.05 kJ/mole and GM-CSF with -606.17 kJ/mole energy bind to the α and β chains of receptor. In the next step the two chains of the receptor were separated and the affinity of each protein to this chains were studied. Based on the results the binding affinity of all three considered proteins to α chain of the protein was weaker than the binding to β chain. The binding energy of interleukin 3, interleukin 5 and GM-CSF to β chain of receptors was -620.37 kJ/mole,-663.80 kJ/mole and -696.07 kJ/mole respectively. According to the results, interleukin 3 and interleukin 5 strongly compete with GM-CSF in binding to Cell SurfaceReceptors on Human Eosinophils.
Keywords: GM-CSF, bioinformatics, docking, interleukin, Receptor, immunology

277. Cloning, Expression, Purification and Production of Antibody against Major Subunit of Coli Surface Antigen 3 as a Candidate Vaccine against Enterotoxigenic *Escherichia coli*
*Alerasool M.S[1], Mousavi S.L[1],Nazarian Sh[2], Bagheri S[1]
[1]Department of Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran, [2]Department of Biological Sciences, Faculty of Sciences, Imam Hossein University, Tehran, Iran

Background: Enterotoxigenic *Escherichia coli* (ETEC) are the major cause of diarrhea. Colonization in the small intestine is thought to be an essential virulence factor of ETEC. Adhesion is mediated by colonization factor antigens. CS3 fimbriae and is one of the most prevalent fimbrial antigens found in clinical isolates. Gene cluster responsible for CS3 biosynthesis include cstA-cstH genes. It has been shown that cstH encodes the major fimbrial subunit and cstA-G encodes the assembly cassette. Therefore, it seems that this subunit has a critical role in attachment of bacteria to epithelial cells of small intestine and this protein is a putative candidate for vaccine development. In the present study, we designed a recombinant protein that could be a suitable vaccine candidate against this pathogen. Materials and Methods: The sequence of CStH encoding gene obtained from Gene bank and omptimized by bioinformatic software. Synthetic gene in pUC57 was sub cloned into pET28a expression vector. Cloning was conformed with restriction digestion analysis. Expression of recombinant protein was performed in E.coli Bl21DE3 and verified with western blots using anti-His antibodies. Purified protein was injected to raise antibody in Balb/c mice. ELISA test carried out on mice sera for determination of antibody production. Results: immunological analyses showed production of high titer of specific antibody in immunized mice. Anti CStH Antibody could bind to CS3 fimbriae and inhibit bacterial attachment to epithelial cells. Coclusion: The recombinant CStH protein can be taken into account as one of the most important components of vaccines candidate against ETEC.
Keywords: Enterotoxigenic *Escherichia coli*, CStH, Vaccine candidate

278. *In Silico* Study and Expression of Truncated Forms of fliC Gene of Enteroaggregative *Escherichia Coli* (EAEC)
*Savar N.S[1], Sardari S[2], Jahanian-Najafabadi A[1], Bouzari S[1]
[1]Department of Molecular Biology, Pasteur institute of Iran, Tehran, Iran, [2]Department of Biotechnology, Pasteur institute of Iran, Tehran, Iran

Background: Enteroaggregative *Escherichia coli* (EAEC) is an emerging cause of acute diarrhea worldwide. It has been shown that flagellin (FliC-EAEC), a major bacterial surface protein of EAEC, activates the innate immunity system via (IL-8) release from several epithelial cell lines. This activation is mediated by Toll-like receptor 5 (TLR-5), which signals through nuclear factor kappa B (NF-κB) which induces transcription of pro-inflammatory cytokines. Based on the ability of FliC-EAEC to activate innate immunity, the flagellin can be considered as a potent adjuvant in designing new vaccines. Materials and Methods: Truncated forms of FliC-EAEC were designed based on its interaction site with TLR-5. The truncated forms were docked to TLR-5 using *Hex* docking server. Depending on the energy values and the pose of their interactions we have chosen the best truncated forms and then, various GST-tag fusions of the truncated forms were investigated for their interaction with TLR-5. Finally, the most appropriate forms were PCR amplified, cloned to pGEX-5X-1 plasmid and expressed by Top10 strain of *E. coli*. Results: Two different amino acid sequences with the most suitable interaction with TLR-5 were obtained following *in silico* analysis of various FliC truncated forms. Cloning of the fragments, fused to the GST-tag of the pGEX-5X-1 was confirmed by DNA sequencing. Finally, expression of the desired fragments were observed on SDS-PAGE and confirmed by western blotting using anti-GST tag specific antibodies. Conclusion: According to our *in silico* results, two truncated forms of the FliC could effectively interact with the TLR-5 receptor. These forms were cloned and expressed in GST fusion forms. The constructs should be purified and further verified by *in vitro* studies. This finding can lead